

Figure 1. Overlap-extension-PCR fragment with purD deletion

Overlap-extension-PCR fragment with recA deletion

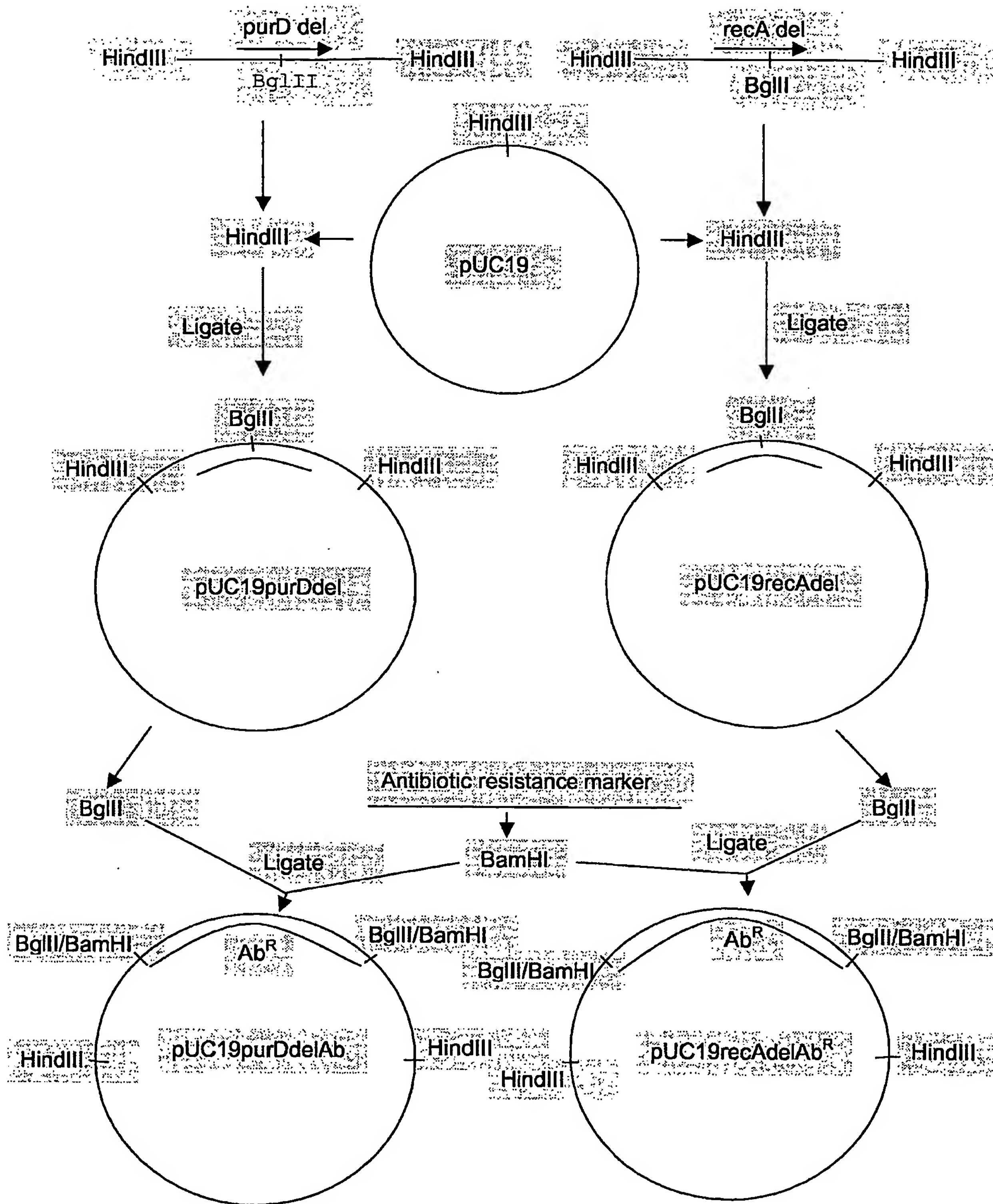


Figure 2A.

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1  GTTCGACCAA ACGGCTTGT GTGCGGTGAA ACATAGCACT CCTTGTGGCG TGGCTTTAGA TGATGATATT TTGCAAGCGT
   >>.....F5.....>>          CTTAAGCTTGGG>>.....F13.....>>
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                                   HindIII

81  ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTGG TGGCATTGTA ACTTTTAATA AAAAAGTAAC AAAAGCAGTG
161  GCAGAAAAAT GTAACGAGAT TTTCCTTGAA ATCGTTGCTG CACCGAGCTT TGAGCCAGAG GCTTTGGAAG TTTTGTCTAA
241  AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GGCGGATTGC
321  TCGTGCAAGA AATCGACAAA TCGTTTAGCA ATGATTTTAA AGTAGTAACC GAAAAACAAC CTACCGAAAA GCAACTTTCT
401  GATTTGGAAT TTGCCATGAA AGTAGTGAAG CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAACCGGAC AAGCTCTAGG
481  CGTGGGCACA GGCGAGACTA ATCGTATTTG GGCAGCACAG CAGGCGATTC AGCGTGCAAA GGAAAAACA CAAGAAAATC
561  TAGTTTGGC TTCCGATGCC TTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT
641  CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT
721  GAGACATTC TTACATTAAA TCAAAAAATC TAAACAATAA TTATCAATAA TTCTAAAACA CAATAAGTAT GAATGCAAAT
                                   >>...purD...>

801  GATTACAAA AAATACTCAT CGTAGGAAAC GGCGCAAGAG AACACGCCAT CGGGTGGAAA ATTAAACAAG ACCACCCTTC
   >.....purD.....>

881  TTGCGAGCTT TTCTTGGCG CAGGAAACGC TGGAACCGAA CAAATTGGAA AAAACATCGT AGCTGAATCT AATTATGGCT
   >.....purD.....>
   <<.....OE-R.....<<AGATCTGGCGCTACGCTAGAAG
                                   -----
                                   BglII

961  TAATGCTTTT TGCTCAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT TGGTAGAAGG TATTGTAGAC
   >.....purD.....>

1041 TTGTTTGAAT CCAATCAATT AAGAATTTT GGTCCAGATA AGCGTGCGGC TAAATTGGAA GGCAGCAAGG CTTTGGCCAA
   >.....purD.....>

1121 AGATTTTATG GAGAAATACG GCGTGCGCAC GGCTTTGCC AAAAGTTTCA ACAATTTTGT AGACGCTAGA GATTATGTAA
   >.....purD.....>

1201 AAGAGCTCAC GCAATCCCT ATCGTGATCA AAGCCAGTGG CTTGGCAGCA GGAAAGGTG TGATCATCGT GCACNTACAA
   >.....purD.....>

1281 CTTGAAGCCG AAACACTTT GCGCAAAATC ATGGAAGACA AAACCTTTGG CGAAGCAGGC AACGAGGTG TAATCGAGGA
   >.....purD.....>

1361 ATACTTAAAA GGTGTGGAAG TTTCTGTGCT TTCTATCTT AACCATAAAG AAATTAAAC TTTCTTGCCT GTAAAAGACC
   >.....purD.....>

1441 ACAAGAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGGG CGGAATGGGC GTAGTGGCTC CTAACCCGCA TTTTACCGAT
   >.....purD.....>

1521 GAGCACATGA AGGAGTTTGA GAAAAACATT TTGCTCCCAA CAAAAAAGG GCTCTTGGCA GAAAAATGC ATTTTGCAGG
   >.....purD.....>

1601 CATTATTTTC TTTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTGG AATACAACAT GCGATTTGGC GACCCAGAAA
   >.....purD.....>

1681 CCGAAGCACT TTTGCCTTTG ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTGAACTT
   >.....purD.....>

1761 AAATGGAAAA ACGAACATGC TTGCTGTGTA GTAATGGCGA GCGGTGGCTA CCCAGGCACT TACGAACTG GTTTTGAAAT
   >.....purD.....>
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1841 CCGAGGATTG AACAAAGTTG ATGTTCCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG  
>.....purD.....>

1921 GGC GCGTGCT CAATGTGGTG GGAAGTGGCG CTACGCTAGA AGAAGCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC  
>.....purD.....>  
GAGATCTGG>>.....OE-F.....>>  
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BglIII

2001 AATTTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAGA TATAATCTCG CTGATTTTTA ACCAAAACAT ATTTAAAAAC  
>.....purD.....>>

2081 GCTTTTGTTA CTTTATATAA CAAAGGCGTT TTTCTATTTT TGTGCCACTA TAACATGATT TAACCCATGA AAAAAATACT

2161 AAAAAATACTC ATTTTCTTAC TGCTCATTC TGGGTTTAT GCCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241 CACAGCTGAG CAATTTATCT TATGGTTTCT CCAGAACACA GCTCGCTTAT GATGAAATTC CGGCTAGTGC TAAATGGGCT

2321 GTAATTGCAG CAGAAGACCA GAATTTTGCC ATTCATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401 CAAAGCGGGC AAGAAATTGC GTGGCGGGAG CACCCTTTCG CAACAACTG CCAAAAATGT ATTTTGTGG CAAGGGCGCA

2481 CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561 TACCTCAACA ATGCCGAAAT GGGCAAAGGC GTTTATGGCA TAGAGGCAGC GGCGCAATAT TATTTAAGA AAAACGCCTC

2641 ACAGCTCACG CCTACCGAGA CGGCACGCAT CATTGCCTGC CTGCCCCATC CCAAAAAATA CAATNTAAAC CCGCCAAGTG

2721 CCTACATCTC AAAACGCGGA CAATGGATTC TCGGCCAAGT GCGAACTTG AAAGGCGATA GGGCTCTGAG CGAGATTGTG

2801 AACACGCCCT AACGCCTGCC TCAACTCTTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAACT CTTCGCACAC

2881 ACTTCCCAA GTCTTTGCAA AGAGTTGGGA GATACTTAGG CACAAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961 CTTAAAAGGA ATAAATAATA ATGTTTTTTA AGCTTTAGGC TTGGCTACTT TTTCAAAGCC TGCTGCCTTC ATGTATCTA  
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HindIII

3041 GGATACGCTT GCCTGGGCGG TAGTTTACGC CTACCTTTTT GATTAAGCC GAATGAAAAT CTTCTCTGT ATCTGCCGCT  
<<.....R8.....<

3121 CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTTGCCCGCT GCCAAGGCGT CTTGAATTAC  
<R8.<<AAGCTTAAG  
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HindIII HindIII

3201 ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCTCG CTGAGTGCCG AAAACTTCTC GATTTGCTTA ACGAGCTGGT

3281 CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTGTGGC TCCCCTGGCT TGCTTGGGT TCTACGCTGA

3361 ATTACATTGT ATTTATGCT CATAATTACT CTATTTTAA TAGCCTCCCG ATGGATATAA AGTTACGCTA CAATTAGGGT

3441 CTCCATAAGC AAATCTATAC CCCTCTCTTT CATATTCCT TCTATTCTT CTTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521 TTAGTCTAT ACCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGAATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601 CCAAGCTCCA TCTGAGCTA TAACATTTGC ATGACATGTA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTGTGTTC

3681 CACCACCACC TCCAGAGCTA CTACTTTTTT TACATTGTCC ATTTTGGTTA GCATGATTTT GTCCGCCATC ACTTACTAAC

3761 TTCTTAGCTT CTGCTAAGGC TTTTCTCTT GCTTCTTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841 CGCTTCTTTT TTATAGTTTA CCGAGGTTCC ATAATAGCCA CTACTACAAT TGTTTCTTGT AAAGTTTTTA TAAAAGATT

3921 GAGTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001 GCGATAGTAT ACGATTGCCC TGCCGGCATT TGTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTAA

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4081 ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTGTAGT GTTAAATCAT TTATATCCCC TCCTTGTCTT TTTGCAGAAG

4161 CTTTGTGTTAC ACTTACAGCA TCATAAGCTC CTTTTCATT GGTATAAGGT ATTTATATGG CCAAAC

Figure 2B.

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1  TAAAGCTGTA AWTGCTATA AACGCCCTTT AGGATAAAAT CTGCCATTTT TTGCAGTATT TTWATAGCTA AAATTTAGAA
   >>.....FrecAOR1.....>>

81  AACACCATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCCACCCT CAATTGATTT GGGCGAATAC
   CTTAAGCTT>>.....F6.....>>
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      HindIII

161  TTGAAATAAA TGGCATCTTC TAGCGACACA TTTTGCAGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAAA
241  WGCTAWTYTT CTGTGTTAAA AAAACTCATA AATTCCTCCA AATATAGAAA TATTCTGTGA AAAGTTGCAA TTTATTAACA
      <<....<

321  CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTTC CGAATCCGAA AGTTTATTTA CGCCCCATCC GATGCCTAGT
   <..FrecA-4...<<

401  CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA
481  ACGAGTACTA GAGTGGTAAA AAGGATTTTT TGACGATTAT TCATGATTTT ATTTTCTCA AAGGTAAATA TTTTAAACCA
561  TAATTCACA AATCTTAAAA TCTATTTAAA TAATAGAGAA ACCAGAAAAA AATCGTATTT TTACGGAATG AATAAAATGT
641  TACAAGTAGG CGATAAAATG CCCGATTTC AAGGTGTAGA CCAATTTGGG AAGGAGCATT CATCTGCCGA TTTCAAAAAT
721  CAGAAATTAG TCGTTTTTTT CTACCCAAAA GCCAGTACGC CAGGTGTCAC GGCAGAGGCT TGCAACATCA ACGATAATCT
801  TGATGCGCTA AAAGCACAAG GCTACCAAGT GATAGGCGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA
881  AATACGATTT TAAATCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTTG GCGTGTGGGG CGAAAAGAAA
961  TTCATGGGTA AAACCTATGA CGGAATTCAT CGTACGACAT TCATTATTGA TGAAAACGGA GTGGTGGAGC GCGTGATAGA
   >>.....F7.....>>
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      EcoRI

1041  AAAAGTGAAA ACAAAGATC ATACCAATCA AATTTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
      >>.....recA.....>

1121  AGGAAAGCAC TCCAGCTAGT GCTTGATAAA ATGGACAAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
   >.....recA.....>
      <<.....OER1.....<

1201  CATAGACGAA AATATTCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
   >.....recA.....>
   <CGAGATCTCGTGGTGGCGT
      -----
      BglII

1281  GTAGAATCGT GGAGATTAC GGTCCAGAAT CTTCTGGTAA AACCACCTTG GCAATTCATG CCATTGCCGA AGCTCAAAAG
   >.....recA.....>

1361  TCTGGCGGAA TTGCAGCTT CATCGATGCA GAGCACGCAT TTGATAGATA TTACGCAGAA AAATTAGGCG TAGATGTTGA
   >.....recA.....>

1441  GCATTTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTTCA GGTGCAATTG
   >.....recA.....>
      -----
      HindIII

1521  ATATTATTGT AATCGATTCG GTAGCGGCTT TAACGCCAAA GTCGGAAATC GACGGAGATA TGGGCGATTC CAAAATGGGA
   >.....recA.....>

1601  TTGCAAGCGC GTTTGATGTC TCAAGCCTTG AGAAAGCTCA CGGGAATAT CAATAAAACC AAATGTACTG CTATTTTCAT
   >.....recA.....>
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1681 CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACTT AATTCTATG  
>.....recA.....>

1761 CATCGGTGCG TCTAGACATT CGTCGTTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG GAAACTTGAC TCGCGTAAAA  
>.....recA.....>

1841 GTAGTGAAAA ACAAGTAGC TCCGCCATTC CGTAGTGCAG AATTCGACAT TATGTATGGC GAAGGAATCT CTAAAGCAGG  
>.....recA.....>  
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EcoRI

1921 CGAGATTTTA GACATTGCTA CCGATTAGA AATCGTGAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAACTAG  
>.....recA.....>

2001 GACAAGGGCG AGATGCCGTG CGTCCGGTAT TGAAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAAGAA  
>.....recA.....>  
CGAGATCT>>.....OEF1.....>>  
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BglII

2081 GAATTAGAGA AAAAATAGAT TTTTAGTTT TTTTAATTAA ACGAAAAATC CGTTCACCTT GTTGAACGGA TTTTATTATG  
>.....recA.....>

2161 CTTGAATGAA TTTATTTCCA ATGGATTGAA TAGCCATGCA CTTTTAAATC TTCGCTATCA TAAGTGATTT CTTGTGCGGT

2241 GTTGGGATAG CAAACTTTAA GTCCTGCGTA TTTGGCAATG GCATGTCCTG CGGCAATGTC CCAAAAGTTT ACAGGTCTAA

2321 AGCGGGTGTA CTCCGTAGCC CACCGATCGG CAATTAGCCC AAGTTTGATA ACGCTTCCCA TAGGCTTTGT GCGGAAAATT

2401 TCATGTTGCG ATTTAATTTT TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAATTG CTACAAAGAA AAGTGTAATC

2481 TTCGGGCAAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAATC CGATTTTCTA GCCATTTTGT

2561 GCAATTGTTG TTGAGTCCCG ATGAATTTAC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641 TACAAACTC CTAGCTTAGC CGTATTATTC TCACTAAGC CTAGACACAC GCAATATTCA TCTGTTTTGT TGACAAAATC

2721 CATGGTGCCA TCAATAGGGT CTGCAATCCA ATAGGTGGGC GTATTTCTAA TTTCTTGTA AGAATCCTTA TCTCCTTCCT

2801 CACTAAAGTA TGGAATGTCT GTAAAGGAAA CATGTTTTTG CAAGATTTTG TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881 GATCCGTCGG CTTTGGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTTAAA ACCTCTTCGC CAGCAAGTGC TACAGCCCGT

2961 GTTGCATTT CTAATAAATT CATAATCATT CTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATTT

3041 TGGCGATAAA AATTAAATTT TATATATAAA ATATCTCTGC AAAAAACCA ATCAAATATT TAGTGAAATA AAAAAATTA

3121 GATTGTAAAT TTGCCTTATG TTTTAGAGA ATACCATAAA TCATAGAAAA AATACGGGCT GGATCGAAGT AATCTGTGGC

3201 TCTATGTTTT CGGGCAAAAC CGAAGAGTTG ATTCGTAGAG TGAAACGAGC CGAATTGGCT GGGCAAAAGG TAGAAATCTT  
<<.....R5.....<<AAGCTTAAG  
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HindIII

3281 TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361 AGGCGAGTTC TAACCTGCCC ATTTTAGCAA GCGATTGTGA TGTGGTGGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441 ATTGTTGAGG TGGCAAATCT TTTAGCTAAT TCGGGGAAAA GAATAATTAT TCGGGGATTA GACATGGATT TTAAAGGTCG  
<<.....RrecAOR1.....<<

3521 TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA

table 5

group	no. of chickens	Treatment			Results	
		vaccination at day 1	challenge at day 25	challenge at day 31	% of max airsac score at day 10 (safety)	% of max airsac score at day 38 (efficacy)
1	25	NDV	NDV	WT-OR aerosol	2.5	25 <sup>b</sup>
2	25	NDV	NDV	WT-OR aerosol	7.5	23 <sup>b</sup>
3	25	NDV	NDV	WT-OR aerosol	68	10 <sup>b</sup>
4	25	NDV	NDV	WT-OR aerosol	0	47
5	25	NDV	NDV	NDV	0	2

<sup>b</sup> Significantly different ( $p < 0.05$ ) compared to the controls (group 11) using two-sided Mann-Whitney U test

table 6

group	no. of chickens	Treatment			Results
		vaccination at day 1	day 30	challenge day 35	
1	15	PurD aerosol	NDV	WT-OR aerosol	no reduction
2	15	NDV PurD aerosol	NDV	WT-OR aerosol	54% <sup>b</sup>
3	15	NDV	NDV	WT-OR aerosol	no reduction
4	15	MAS	NDV	WT-OR aerosol	no reduction
5	15	MAS PurD aerosol	NDV	VT-OR aerosol	50% <sup>b</sup>

<sup>b</sup> Significantly different ( $p < 0.05$ ) compared to the controls (group 11) using two-sided Mann-Whitney U test